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Deconvoluting lung evolution: from phenotypes to gene regulatory networks

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Synopsis Speakers in this symposium presented examples of respiratory regulation that broadly illustrate principles of evolution from whole organ to genes. The swim bladder and lungs of aquatic and terrestrial organisms arose independently from a common primordial ''respiratory pharynx'' but not from each other. Pathways of lung evolution are similar between crocodiles and birds but a low compliance of mammalian lung may have driven the development of the diaphragm to permit lung inflation during inspiration. To meet the high oxygen demands of flight, bird lungs have evolved separate gas exchange and pump components to achieve unidirectional ventilation and minimize dead space. The process of ''screening'' (removal of oxygen from inspired air prior to entering the terminal units) reduces effective alveolar oxygen tension and potentially explains why nonathletic large mammals possess greater pulmonary diffusing capacities than required by their oxygen consumption. The ''primitive'' central admixture of oxygenated and deoxygenated blood in the incompletely divided reptilian heart is actually co-regulated with other autonomic cardiopulmonary responses to provide flexible control of arterial oxygen tension independent of ventilation as well as a unique mechanism for adjusting metabolic rate. Some of the most ancient oxygen-sensing molecules, i.e., hypoxia-inducible factor-1alpha and erythropoietin, are up-regulated during mammalian lung development and growth under apparently normoxic conditions, suggesting functional evolution. Normal alveolarization requires pleiotropic growth factors acting via highly conserved cell–cell signal transduction, e.g., parathyroid hormone-related protein transducing at least partly through the Wingless/int pathway. The latter regulates morphogenesis from nematode to mammal. If there is commonality among these diverse respiratory processes, it is that all levels of organization, from molecular signaling to structure to function, co-evolve progressively, and optimize an existing gas-exchange framework.

Introduction

In order to appreciate lung evolution, we should appreciate the spectrum of phenotypes and their relationship to the underlying molecular and genetic regulatory networks. In this symposium, some interesting examples of respiratory regulation, ranging from whole organ to genes, to illustrate general principles of evolution were presented.

All gas exchange organs share a number of structural properties, as illustrated below, but they are not identical. The differences, however, can only in part be explained by historical, evolutionary biology, or by comparative, and/or developmental studies. Functional analysis of lung structure, compared with physiologically measured gas exchange, can help explain incongruities. In addition, since gas-exchange organs are integrated with the circulatory system, knowledge of the structure and function of the heart helps evaluate the whole gas-exchange strategy. Even taken together, however, comparative studies do not reveal the proximate mechanisms that result in the observed phenotype. We, therefore, take a look at some such mechanisms at the genomic level. While certainly not exhaustive, these examples serve to illustrate some of the mechanisms by which adapted phenotypes may develop. We begin with an overview of structural types of vertebrate gas-exchange organs.

This paper summarizes one of the 22 symposia that constituted the ''First International Congress of Respiratory Biology'' held August 14–16, 2006, in Bonn, Germany.

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Comparative functional design of the vertebrate respiratory organs with particular emphasis on the avian lung[4]

Within a robust cladistic framework, structural changes occurred that suggest similar underlying genetic mechanisms: the air spaces became smaller (Daniels and Orgeig 2003), the air-blood barrier thinned (Meban 1980), and the walls of the terminal respiratory units stabilized in the most highly derived groups.

The flux of respiratory gases occurs by diffusion along gradients of partial pressure. Accordingly, the physical properties of the gas-exchange organs are consistent with optimization of conditions for diffusion and they also reflect the physical properties of water or air, the only two naturally occurring respiratory media. Consequently, gills are broad and relatively coarse evaginations, accessible through wide openings, whereas lungs are invaginations that may contain very small airspaces and are accessible through narrow tubes (trachea, bronchi).

Morphologically, gas exchangers present remarkably diverse phenotypes (Maina 2002). They also share many structural and functional properties. These include a large respiratory surface area, a thin blood-gas/water barrier, and intense vascularization of the respiratory surface. The specific functional designs of the respiratory organs/structures correlate generally with the metabolic demands of the particular animals, and with factors such as body mass, habitat, sex, age, and lifestyle (Maina 2002).

Lungs, however, are ventilated tidally, which introduces a constraint imposed by dead space. The disadvantages of dead space have been balanced in single-chambered lungs of snakes and many lizards by a large central lumen that supplies air to the faveoli between breathing episodes. In mammals, this is also true of the alveolar ducts and sacs, true dead space being present in the bronchial air-conduits, which evolved from the chambers of the much simpler multichambered ''reptilian'' lung.

In the avian respiratory system, the lung (the gas exchanger), has been totally disengaged from the ventilator, the air sacs. The relatively small, constant-volume lung is ventilated in a caudo-cranial direction by the synchronized, bellows-like action of two sets of air sacs between which it is intercalated. Thus, although breathing in birds is tidal, the paleopulmonic parabronchi (the site where much of gas exchange occurs) are ventilated unidirectionally and continuously. Utilizing diverse structural and functional refinements, the lung-air sac system of birds is the most efficient respiratory apparatus found among the air-breathing vertebrates (Powell and Hopkins 2004).

From this general basis, we proceed to investigate a fascinating incongruity between the structure and the function of lungs. Many mammals display a much greater morphometrically estimated diffusing capacity than they physiologically use, while others in the same class of body mass exploit a much greater proportion of their diffusing capacity. We present here a model that not only explains this apparent contradiction but also represents a new approach to the functional interpretation of lungs in general.

Lung design and efficient gas exchange: lessons from comparative mammalian physiology and morphometry[5]

Evolution of the mammalian lung has taken a different track than that of the avian lung in that it forms a gas exchanger in the end part of a branched airway tree. This may appear to be a less efficient design than that of the bird lung, but it does have other advantages such as the facilitation of acid–base regulation of the internal milieu through the regulation of CO_2 discharge.

An important question is whether the mammalian lung achieves functional efficiency and adequacy through the matching of the design of its structural apparatus, gas-exchange tissues, airways, and blood vessels, with the functional demands of the organism. In that sense, the lung is an essential part of the pathway for oxygen that leads to the sites at which energy is required, the mitochondria in the cells (Weibel 2000). The largest demand for O_2 occurs when the locomotor muscles are fully active in exercise, so we hypothesize that the lung's gas exchanger is quantitatively matched to the maximal O_2 consumption (Weibel and Hoppeler 2000), a value that varies greatly with both body size and physical capacity among different mammalian species, (Weibel 2000). Studying the relationship between lung design and O_2 needs among different mammalian species may offer some insights into the factors, genetic or epigenetic, that adapt the structural design in this vital organ to the variation in functional demand imposed by habitat and life style.

In the mammalian lung, the gas exchanger at the periphery of a closed-bag system is ventilated through a single port by to-and-fro air flow. On inspiration, the residual air near the gas exchange surface in the alveoli is thus replenished with O_2 that is continuously transferred to the blood during gas

exchange. The pulmonary gas exchanger is characterized by a very large surface of air-blood contact, nearly the size of a tennis court in humans (120 m^2), a very thin tissue barrier (~1 μm); and a large capillary blood volume (200 ml in humans), all of which determines the pulmonary diffusing capacity DL_{O2} (Weibel 2000; Weibel and Hoppeler 2000).

Comparing different mammalian species, we find DL_{O2} about matched to the body's maximal O_2 needs as induced by exercise, but not in a simple fashion. We find that in "athletic" species, such as dogs, horses, or antelopes, DL_{O2} is well matched to maximal O_2 needs, whereas "normal" species—including humans—have about 50% excess diffusing capacity. When studying animals of different body size, we find DL_{O2} to be proportional to body mass (M), whereas maximal O_2 consumption scales with $M^{0.87}$. As a result, small mammals have a DL_{O2} that matches O_2 needs, whereas larger animals have excess DL_{O2} that increases with body mass. Why is this? One possibility is that replenishing O_2 in the alveoli may be more difficult in larger lungs.

The problem of supplying oxygen to all points on such a large surface was solved by the evolution of an airway tree with alveoli arranged around the last few generations, thus sequestering the alveolar surface in the pulmonary acini (Weibel et al. 2005). Pulmonary arteries and veins branch in parallel with the airways and penetrate into the acinus for perfusion of the capillary networks associated with the alveoli. Because of the arrangement of alveoli along the last branches of the airway tree, alveoli are ventilated in series, whereas the units of the capillary network are perfused in parallel. In the acinus, replenishment of O_2 at the alveolar surface occurs first by convective air flow, and then by diffusion of O_2 in the air. This is combined with continuous absorption of O_2 at the alveolar surface, beginning with the first alveolus on the airways, which removes O_2 from the acinar air, a phenomenon called screening. The problem, therefore, is provision of enough oxygen for the final generation of the branching acinar tree where half the gas-exchange surface occurs. Adequate supply of oxygen depends on the size of the acinus and the dimensions (diameter and length) of the acinar airways, as well as on the permeability of the barrier. The size of the acini increases with body mass so that large species have larger acini with longer path length from the entrance to the end. Screening is therefore more pronounced in these large lungs, which may explain why they need a larger, seemingly excessive, diffusing capacity (Weibel 2000; Weibel and Hoppeler 2000; Weibel et al. 2005).

In the preceding section, one comes to appreciate how a highly derived respiratory organ, namely the mammalian lung, can display functionally meaningful diversity. In terms of the metabolic spectrum of vertebrates in general, however, mammals are high-performance organisms. Where did lungs originate and how did the structural types that we see arise?

Origin and early radiation of vertebrate lungs[8]

The path of lung evolution may be deduced from lung development to the extent that ontogeny recapitulates phylogeny. In order to exploit this premise, we must first establish the homology of all vertebrate lungs, a question that is still unresolved. Both the lung and the swimbladder are evaginations of the gut tube, suggesting that they may have common structural origins, and they both regulate gas exchange with the environment. Therefore, they may share common genetic origins. In addition, there is no doubt that the lungs and the swimbladder both are derivatives of the posterior pharynx and as such are homologous.

The hypothesis—based on extant phylogenetic bracketing—that Osteichthyes originally possessed paired ventro-lateral pharyngeal pouches that could have given rise to lungs is supported by embryological data. There is no embryological evidence, however, to support the migration hypothesis, by which the paired ventral lungs are presumed to have formed the unpaired, dorsal swimbladder (Moser 1904). The most plausible hypothesis is that both have their origins in a "respiratory pharynx" (Neumayer 1930; Wassnetzov 1932). Thus, it is unlikely that lungs and the swimbladder are derived one from the other, since none of the three homology criteria (same relative location, same embryological origin, ontogenetic, or phylogenetic continuum) used in comparative anatomy and evolutionary biology are satisfied. Even the discovery of activity of the same genes in lung and swimbladder development would not prove the homology of the organs: hand and foot are not homologous although there genetic patterns of development are similar.

In lungfish—unlike tetrapods—the left lung receives its nervous and blood supply from the right side: the reverse applies to the right lung (Marcus 1937). Therefore, it is unlikely that tetrapod lungs evolved directly from those of lungfish: more probably both are derived from a common ancestral condition that is more similar to that of

lissamphibians. In that group, lungs originate bilaterally from the 7th or 8th gill pouches. This is even true for gymnophionans in which adults have a single lung (Marcus 1923). In amniotes, however, lungs originate through bifurcation of the unpaired laryngotracheal pouch as in lungfish, but unlike lungfish the innervation and vascular supply is ipsilateral.

Although it is tempting to assume that the first amniotes had single-chambered lungs resembling those of lissamphibians, it is equally probable that the organs had a simple multichambered structure, reminiscent of that in lungfish (Hughes and Weibel 1976). Single-chambered lungs are present in all scincomorph lizards and Gekkota, whereas in phylogenetically more basal Iguania the central lumen characteristically is interrupted by a small number of large septa (Perry 1998). The multichambered lungs of turtles are reminiscent in their branching pattern of mammalian lungs, and numerous similarities also exist between the basic Bauplan in birds and crocodilians (Perry and Sander 2004). This allows approximation of lung structure in pterosaurs and dinosaurs by phylogenetic bracketing. Multichambered lungs appear to have evolved in platynotan lizards separately from other sauropsids (Perry 1998).

Apart from the superficial similarity in branching pattern shared with turtle lungs, mammalian lungs appear to have taken a quite different evolutionary path from those of other amniotes. The compliance of the broncho-alveolar mammalian lung is more than an order of magnitude less than that of the stiffest sauropsid lung (Perry and Duncker 1980; Klein et al. 2003).

The origin and evolution of the broncho-alveolar lung is conceivable if we consider the gastralia of early ancestors of mammals to have served in (1) preventing paradoxical movement of the abdominal wall during inspiration and (2) permitting the inflation of a stiff lung. A stepwise functional replacement of the gastralia by the diaphragm can be envisioned, resulting in the coupled evolution of the low-compliance lung/diaphragm complex we know in mammals.

The lungs form together with the heart and blood vessels a structure-function complex (faculty). This cardio-respiratory faculty, together with the respiratory musculature and the central nervous control constitute the respiratory apparatus. We summarize here the major cardiovascular components that occur in tetrapods and point out their correlation with different lung types.

Patterns and processes in the evolution of the vertebrate cardiopulmonary system[2,3]

Trying to understand vertebrate evolution by analyzing modern species is literally reasoning after the fact. This has been a major obstacle to the effective study of evolutionary biology. One way in which to avoid this inherent pitfall is to compare lung evolution with that of the heart, since these two organs must have evolved as a single functional unit. Comparison of the structural and functional similarities and differences in the coupled evolution of lung and heart among phyla might provide insights into commonly evolved gene-regulatory networks.

The primary function of the cardiovascular system in all animals is to provide an adequate supply of oxygen and nutrients to the tissues, while ensuring the removal of carbon dioxide and other metabolic waste products. In vertebrates, this goal is achieved through circulatory and cardiac designs that are commonly portrayed as an evolutionary progression from the serially-arranged chambers of the fish heart to the completely divided four-chambered heart of birds and mammals (Burggren 1987; Hicks 1998, 2002).

The four-chambered heart, which provides complete separation of the systemic and pulmonary circulations, seems to have evolved independently in two lineages, one that gave rise to mammals and the other within an ancestral group of ''reptiles,'' the archosaurs, that gave rise to modern birds and crocodiles. Historically, the hearts of lissamphibians and ''reptiles'' were viewed as intermediate phylogenetic steps; functionally inefficient compared to the ''perfect'' circulation of birds and mammals. It is now recognized, however, that the unique morphologies of the ''reptilian'' and lissamphibian hearts, combined with autonomic regulation of heart rate and cardiac contractility as well as control of pulmonary and systemic vascular resistances, provides a flexibility of cardiopulmonary responses not possible in birds and mammals (Burggren 1987; Wang and Hicks 1996a; Hicks 1998, 2002). In particular, the ability to by-pass the pulmonary circulation [right-to-left (R–L) cardiac shunt] provides the ability to regulate arterial PO_2 independent of lung ventilation (Wang and Hicks 1996b; Wang et al. 1997).

Lissamphibians and ''reptiles'' regulate the magnitude and direction of their cardiac shunts and recent studies show that the magnitude and net direction of the cardiac shunts correlate with overall oxygen demands (Wang and Hicks 1996b, 2002).

For example, in the turtle, *Trachemys scripta*, the development of a R–L shunt and the subsequent reduction in systemic oxygen transport, may trigger a significant hypometabolism (Platzack and Hicks 2001; Wang and Hicks 2002). Thus, large R–L cardiac shunts are characteristic for a variety of different lissamphibians and ''reptiles'' at resting, undisturbed conditions and may contribute to a reduction in overall metabolic demands. This could be particularly important in semi-aquatic species in which an increase in R–L shunt is often associated with diving (Wang and Hicks 1996a).

In contrast, situations with increased oxygen demands, such as locomotor activity, digestion or higher body temperatures, are associated with prompt reductions in R–L cardiac shunting (e.g., 21–23). This reduction in the R–L cardiac shunt increases arterial oxygen levels and provides an important means of augmenting systemic oxygen delivery (Wang and Hicks 1996b; Wang et al. 1997). In many cases, it seems that the R–L cardiac shunt can be completely abolished and the undivided hearts of ''lower'' vertebrates, therefore, can effectively separate venous and arterial blood; in terms of arterial oxygen delivery, these animals have functionally divided the undivided. As a consequence, cardiac shunts may not pose a significant limitation to oxygen transport in these vertebrates.

While the degree of cardiac shunting can be reduced, or even abolished, during periods of high metabolic demands, the anatomically undivided heart functions as a single pressure pump and can not separate blood pressures between the systemic and pulmonary circulations. The hemodynamic consequences of this anatomy expose the lungs to the detrimental effects of high pulmonary blood pressures. A few groups of ''reptiles,'' namely varanid lizards and pythons, however, have circumvented this constraint. In these species, physical activity and/or digestion are associated with mammalian-like rates of oxygen consumption. Although the heart in these species, as in other noncrocodilian ''reptiles,'' is anatomically undivided, a well-developed partial ventricular septum (muscular ridge), effectively separates the ventricle during systole. Thus, during cardiac contraction, the muscular ridge presses against the dorsal wall of the ventricle resulting in mammalian-like pressure separation between the pulmonary and systemic circulations (Burggren and Johansen 1982; Wang et al. 2003). Comparative studies suggest that the evolution of the anatomically divided ventricle in birds and mammals may have been correlated with the high oxygen demands associated with endothermy and the need to protect the lungs from the edema that would result from high pulmonary blood pressures.

The developmental process of the cardiorespiratory faculty does not end with the birth of the animal. On the contrary, certain compensatory possibilities remain open even in the mammalian lung. Which are these and how are the signals transmitted to the genome?

Signals for compensatory lung growth[6]

Any insult that causes the loss of functioning lung units imposes signals that—when sufficiently strong—elicit coordinated activation of multiple metabolic pathways leading to accelerated cellular growth and tissue remodeling, and ultimately to preserving or improving lung function. If lung function is not improved, then the growth/remodeling is not compensatory. Pneumonectomy is a robust model of compensatory lung growth because it imposes signals for balanced growth of the remaining uninjured alveolar units, resulting in enhancement of gas-exchange capacity (Takeda et al. 1999). Two main signals are thought to initiate compensatory growth after pneumonectomy: mechanical forces and alveolar hypoxia (American Thoracic Society Workshop Document 2004).

During normal growth, mechanical forces acting on the lung and thorax are interdependent; the rate of growth and final size of the lungs are constrained by that of its container. After one lung is removed, intrathoracic pressure causes the remaining lung to expand to nearly twice its normal size and its blood flow doubles due to the redirection of cardiac output. The resulting alveolar-capillary stress and strain is believed to be a major signal that induces cellular pathways of adaptation. Transmission of the mechanical signals, however, is nonuniform owing to the presence of asymmetric ligaments and mediastinal structures (Ravikumar et al. 2004). Preventing expansion of the remaining lung by the use of a space-occupying prosthesis impairs compensatory response (Wu et al. 2000; Hsia et al. 2001); subsequent deflation of the prosthesis leads to progressive improvement of lung function. Mechanical factors can account for about 70% of the compensatory response. The remainder of the observed response is attributed to other factors such as intermittent alveolar hypoxia during exercise and various paracrine growth factors.

Chronic hypoxia stimulates erythrocyte production by stimulating proliferation and by inhibiting the apoptosis of erythrocyte progenitor cells (Jelkmann and Metzen 1996). Chronic hypoxia also

stimulates alveolar growth in developing animals (Hsia et al. 2005). We hypothesized that these two seemingly independent actions of hypoxia are mediated at least partly via the same pathway, namely erythropoietin (EPO). EPO receptors are present in lung tissue and its expression is regulated during postnatal development (Foster et al. 2004). Since the normal postnatal lung is not hypoxic this observation suggests that EPO signaling responds to nonhypoxic factors as well, for example, mechanical forces. Indeed, we find that EPO signaling is upregulated during lung growth after pneumonectomy in the absence of overt hypoxemia. In addition, the transcriptional regulator of EPO, hypoxia-inducible factor-1alpha (HIF-1α), is upregulated during these two types of lung growth (Zhang et al. 2006). Mechanical forces have also been reported to induce HIF-1α-targeted signaling in vitro in the myocardium and skeletal muscle (Chang et al. 2003). From an evolutionary perspective, the principle of metabolic economy is conserved when independent signals (hypoxia and mechanical forces) utilize common regulatory pathways towards achieving the same goal (balanced compensatory alveolar growth) instead of each utilizing a separate pathway.

Let us now step outside the familiar vertebrate respiratory paradigm to a completely different system, namely the nematode vulva. Here, we can see how fickle developmental processes are and how the same signals in closely related organisms can result in completely different phenotypes.

Lessons from an unrelated system. Evolution of nematode vulval development: drastic changes of signaling networks in a conserved developmental system[7]

The recognition that interactions of ligands and receptors were a key to physiological processes began with the discovery of second messengers for hormones by Sutherland in the 1960s (Sutherland 1972). That concept was then applied to the mechanism of action of growth factors (Cohen 1983). Such physiological ''nodes'' provide an example of the ''middle out'' approach to the deconvolution of Systems Biology (Brenner 1998). That is to say, determining physiologic mechanisms based on cell–cell signaling mediated by growth factors and their receptors across phyla, development, aging, and repair (Torday and Rehan 2004) will elucidate the unifying evolutionary processes that formed them initially.

Cell–cell signaling and specification of cell fate have been well studied in the vulval development of *Caenorhabditis elegans* and provide a paradigm in evolutionary developmental biology (Pires-daSilva and Sommer 2003). Homologous precursor cells form vulval tissue in all studied species, but the underlying cell–cell interactions changed dramatically during nematode evolution. In *Pristionchus pacificus*, compared with *C. elegans*, for example, genetic and molecular analyses indicate fundamental differences in the signaling networks that control vulval formation. Wingless/int (Wnt) pathway mutations result in a vulvaless phenotype in *C. elegans*, but in a multivulval *Pristionchus pacificus*. Future developmental studies of different strains of *Pristionchus* and of closely related species will identify the micro-evolutionary steps in Wnt signaling.

Meanwhile, back in vertebrates, we see similar roles of substrate–ligand interactions in developmental signaling. Could these interactions provide the key for a better understanding of developmental phases and mechanisms in the evolution of lung structure?

Deconvoluting lung evolution by "Haeckling" functional genomics[1]

Ligand–receptor signaling involving parathyroid hormone-related protein (PTHrP) is crucial for the development and proper functioning of lungs in all vertebrates studied. Its expression correlates with lung maturation, homeostasis, and repair as well as alveolar size, septal thickness and composition of the matrix (Torday and Rehan 2004). Since these are important variables in lung evolution and development, PTHrP–ligand interaction may be a model of gene-regulated networks that mediate evolution in vertebrate lung.

PTHrP, a highly conserved, stretch-regulated protein, is unusual among the paracrine growth factors that have been identified as mediating lung development because: (1) the mouse PTHrP gene knock-out is stage specific and results in failed alveolarization, (2) PTHrP is expressed in the endoderm and binds to the mesoderm, and (3) only PTHrP has been shown to act pleiotropically to integrate surfactant synthesis and alveolar capillary perfusion, i.e. alveolar homeostasis.

In addition, PTHrP signaling from the epithelium to the mesoderm is highly significant—it is the earliest known developmental signal emanating from the endoderm, perhaps reflecting its atavistic, evolutionary relationship to the swim bladder as an adaptation to gravity (Torday 2003). In contrast

to other research groups that have concentrated on epithelial–mesenchymal trophic units and the role of the fibroblasts scaffold in regulating local inflammatory responses, we have demonstrated the dependence of the fibroblast phenotype on epithelially-derived PTHrP. Thus, PTHrP appears to have both an ancient and a continuing central role in lung differentiation, function, and possibly evolution.

A possible central role for PTHrP in lung ontogeny and evolution

PTHrP turns off myofibroblast differentiation by inhibiting Gli, the first molecular step in the mesodermal Wnt-β catenin pathway (see above), and also induces formation of fibroblasts (Vaccaro and Brody 1978). The latter cell-type is found in the adepithelial interstitium next to type-II cells in the lungs of amniotes, including both newborn and adult humans. It is characterized by neutral lipid inclusions wrapped in adipocyte differentiation related protein (ADRP) that mediates the uptake and trafficking of lipid from the lipofibroblast to the type-II cell. There it is used in the synthesis of surfactant phospholipid and protects the alveolar acinus against oxidant injury. The concomitant inhibitory effect of PTHrP on growth of both fibroblasts and type-II cells, in combination with augmentation of surfactant production and up regulation of type-IV synthesis of collagen, would have the net effect of distending the thinning alveolar wall, thus further up-regulating PTHrP and physiologically stabilizing the thin, interalveolar septa. This process could have been a key step in the origin of the mammalian broncho-alveolar lung from a multichambered ''reptilian'' precursor (see above).

During alveolarization, the formation of fluid in the lung up-regulates the PTHrP signaling pathway in the endoderm, causing the down-regulation of the Wnt/β-catenin pathway, finally leading to the differentiation of the lipofibroblast (Torday and Rehan 2006). These cells dominate the alveolar acinus during lung development in the fetus, but are highly apoptotic in the post-natal lung during the active phase of alveolarization and remodeling of the alveolar wall. Central to this paracrine determination of the mesodermal cell-types is the failure of the fibroblasts to terminally differentiate. As a result, they remain ''plastic.''

The swimbladder of fish and the interstitium of the frog lung are characterized by myofibroblasts, lipofibroblasts (like alveolar type-I and type-II cells) being seen first in amniotes. The appearance of myofibroblasts rather than lipofibroblasts during injury and repair of the amniote lung is consistent with a reversion to a genetic program that is typical of an earlier vertebrate phylogenetic stage. By viewing this process as the equivalent of ''evolution in reverse'' (Teotonio and Rose 2001), we can potentially devise strategies to drive the lung back towards the more plastic amnioteic phenotype, thereby reversing the detrimental effects of maladaptive disease states based on evolutionary molecular biological principles for the first time.

Conclusion

This symposium highlights the astounding variety of distinct morphological phenotypes displayed by vertebrate lungs. The patterns of structure and function seen throughout development are the ultimate result of millennia of natural selection in response to ever-changing environmental pressures as well as the needs of the organism, a dynamic process that is on-going. The genetic control of developmental events has at its disposal the genetic toolbox that has evolved from previous epochs of biological existence. Interestingly, during healing or compensatory growth of the lung there is often a reversion to a genetic program that is typical of an earlier phylogenetic stage, suggesting that comprehensive knowledge of the genetics of wound healing and ontogeny could hold the key to a better understanding of the past biologic history, present phenotype, and future direction of evolution of the lung.

The symposium also highlights the fact that the lung does not evolve independently, but is directly influenced by co-existing selective pressures on the circulatory system and on other organs of oxygen transport and metabolism that can alter the fitness of the adult animal. We are still far from uniting proximate ontogenetic mechanisms and ultimate adaptive processes to explain the evolution of lung structure and function, but efforts to forge closer communication, as well as collaboration, among evolutionary biologists, comparative morphologists, physiologists, developmental biologists, and clinician-scientists at all organizational levels, exemplified by this symposium, points the way to a promising future.

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